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Preliminary Observations on Flower Visitors of *Lespedeza* Subgenus Macrolespedeza in Korea

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Flower visitors of four species of *Lespedeza* subgen. Macrolespedeza were observed in Korea for the first time. The major flower visitors were apoid bees, especially *Apis, Tetralonia, Megachile*, and *Bombus*. They showed a considerable degree of flower constancy to Macrolespedeza judging from analyses of pollen loads, hence they were considered to be important pollinators for Macrolespedeza. Significant differences were not observed in flower visitor fauna among the species except *L. melanantha* which represented predominance of *Bombus*. Although the predominance of *Bombus* has not mentioned so far in *Lespedeza*, it was the most important pollinator for *L. melanantha*.

Key words: flower visilor, Macrolespedeza, apoid bee, pollinator, flower morphology

The genus *Lespedeza* Michx. (Leguminosae) is composed of about 40 species distributed mainly in temperate regions of Asia and eastern North America. It has been classified into two subgenera, Macrolespedeza and Lespedeza (Ohashi 1982). Subgenus Macrolespedeza comprising 11 species is confined in East Asia (Akiyama 1988) and has been distinguished from subgenus Lespedeza mainly in lacking cleistogamous flowers. Chasmogamous flowers of Macrolespedeza are larger than those of subgen. Lespedeza. (We hereafter call the species of subgenus Macrolespedeza in the genus *Lespedeza* treated in this paper simply Macrolespedeza or Macrolespedeza species.) These features in reproduction may suggest that Macrolespedeza species is necessary to attract pollinating insects for their sexual reproduction. In general, these insects play important role for diversification of floral characters and the case of Macrolespedeza species should not be an exception. However, we could not confirm the importance of pollinating insects because no extensive comparisons have been made so far about pollinating insect fauna of each species of Macrolespedeza. Here we report results of preliminary observations of flower visitors on Macrolespedeza species during our field expedition to Korea in 1997. This is the first report focusing on the comparisons of flower visitor fauna of Macrolespedeza species, although further observation should clarify a particular relationship between Macrolespedeza species and flower visitor fauna.

Materials and Methods

Field observation was carried out from 1st to 9th in September, 1997, in Korea. The investigation sites and observed Macrolespedeza species were listed in Table 1. Although five species are known in Korea (Akiyama 1988), we observed following four

Table 1. Dates, sites with abbreviation, and *Lespedeza* species observed in this study. Numbers in parenthesis indicate individuals observed

- Sept. 1, 1997. Mt. Kwanak, Kwanak-gu, Seoul (KWA)
  - *Lespedeza cyrtobotrya* (17)
- Sept. 3, 1997. Hwaom Temple-Nogodan, Kurye-gun, Chollanam-do (NOG)
  - *Lespedeza bicolor* (1)
  - *Lespedeza formosa* subsp. *velutina* (5)
- Sept. 4, 1997. Nogodan-Panyabong, Kurye-gun, Chollanam-do (PAN)
  - *Lespedeza melanantha* (2)
- Sept. 5, 1997. Namhae-gun, Kyongsangnam-do (NAM)
  - *Lespedeza cyrtobotrya* (12)
  - *Lespedeza formosa* subsp. *velutina* (2)
- Sept. 7, 1997. Mt. Chongyang, Inchon (CHO)
  - *Lespedeza bicolor* (11)
  - *Lespedeza cyrtobotrya* (21)
  - *Lespedeza formosa* subsp. *velutina* (1)
- Sept. 9, 1997. Mt. Taejok, Talsong-gun–Kyongsan-gun, Kyongsangbuk-do (TAE)
  - *Lespedeza cyrtobotrya* (1)
  - *Lespedeza formosa* subsp. *velutina* (4)

species, *Lespedeza bicolor* Turcz., *L. cyrtobotrya* Miq., *L. formosa* (Vogel) Koehne subsp. *velutina* (Nakai) S.Akiyama et H.Ohba, and *L. melanantha* Nakai. The remaining one, *L. maximowiczii* Schneid. was already over its flowering when we found it in native habitats. When we found flower visitors on plant individuals of these species, we observed the plants more than three minutes and recorded all visitors. Hymenopteran insects except Eumenidae were identified at genus level and other insects at family level. All voucher specimens of Macrolespedeza species are deposited in the Herbarium, Biological Institute, Graduate School of Science, Tohoku University (TUS) and insect specimens are kept in the Biological Institute, Graduate School of Science, Tohoku University and some *Bombus* specimens in the Sapporo Institute of Technology.

To examine constancy of flower visitors to definite flowers, proportion of pollen grains of Macrolespedeza for those of other plant groups in pollen loads were examined for the sampled specimens. Pollen grains were removed from corbicula (Apidae) or scopa (other family) of 18 individuals of bees and suspended in 500 μl of 70% ethanol. We removed 25 μl of the suspended solution and counted pollen grains include in it under a light microscope. Pollen grains were compared with samples directly removed from flowers and classified into two categories, i.e., pollen grains of Macrolespedeza or those of other plants.

Results

Flower visitors observed on plants of Macrolespedeza were listed in Table 2. In every study sites, 81.8–100% of total visits to the flowers were apoid bees (superfamily Apoidea, Hymenoptera). Examined pollen loads of all but two samples of apoid bees contained high proportion of Macrolespedeza pollens (83.8–100%: Table 3). This fact suggests a high degree of flower constancy of bees. Major pollinators of Macrolespedeza are, therefore, apoid bees as in other papilionaceous legume species (Leppik 1966, Arroyo 1981).

Among apoid bees, *Apis* (Apidae: 38.6% of total observations), *Tetralonia* (Antho-

Table 2. Visitors of Macrolespedeza observed from 1st to 9th in September, 1997

		No. of flower visitors						
		Hymenoptera						
		Apidae		Anthophoridae			Megachilidae	
Macrolespedeza species	Study site	*Apis*	*Bombus*	*Tetralonia*	*Xylocopa*	*Thyreus*	*Megachile*	*Coelioxys*
Lespedeza bicolor	NOG						3	
	CHO	12		7			1	3
Lespedeza cytobotrya	KWA	32	2	1			7	
	NAM	7	20	22			3	
	CHO	40		6	1		1	
	TAE					1	1	
Lespedeza formosa ssp. *velutina*	NOG						7	3
	NAM			4			1	
	CHO							
	TAE			3			9	
Lespedeza melanantha	PAN		5				4	

		No. of flower visitors					
		Hymenoptera			Diptera	Lepidoptera	
		Halictidae		Eumenidae	Syrphidae	Lycaenidae	Hesperiidae
Macrolespedeza species	Study site	*Nomia*	*Lasioglossum*				
Lespedeza bicolor	NOG						
	CHO	7	1				
Lespedeza cytobotrya	KWA						
	NAM			2		1	5
	CHO	4				2	1
	TAE						
Lespedeza formosa ssp. *velutina*	NOG		2	1			
	NAM						
	CHO	1					
	TAE	1					
Lespedeza melanantha	PAN				2		

phoridae: 18.2%), *Megachile* (Megachilidae: 15.7%), and *Bombus* (Apidae: 11.4%) were frequently observed in our sites. *Apis* was the dominant visitor at KWA (76.2% of total visits) and CHO (59.8%), but both sites were close to an urban area and all sighted *Apis* were naturalized European honeybees (*A. mellifera*). In NAM, a native honeybee (*A.

Table 3. Proportion of Macrolespedeza pollen in pollen loads of flower visitors

Bees	Study site	*Lespedeza* species	Macrolespedeza pollens (%)
Apis	KWA	*L. cyrtobotrya*	100
	CHO	*L. bicolor*	99.5
Bombus	KWA	*L. cyrtobotrya*	99.7
	NAM	*L. cyrtobotrya*	100
	NAM	*L. cyrtobotrya*	91.1
Tetralonia	KWA	*L. cyrtobotrya*	100
	NAM	*L. cyrtobotrya*	100
	NAM	*L. cyrtobotrya*	99.7
	CHO	*L. cyrtobotrya*	97.7
	TAE	*L. formosa* subsp. *velutina*	83.8
Megachile	KWA	*L. cyrtobotrya*	100
	KWA	*L. cyrtobotrya*	97.9
	NOG	*L. formosa* subsp. *velutina*	29.0
	CHO	*L. bicolor*	32.3
	TAE	*L. formosa* subsp. *velutina*	99.8
Nomia	CHO	*L. cyrtobotrya*	99.8
	CHO	*L. cyrtobotrya*	100
	CHO	*L. formosa* subsp. *velutina*	99.8

cerana) was observed on flowers of *Lespedeza cyrtobotrya* though the proportion was relatively low among the other apoid genera (11.7% of total visits compared with *Tetralonia* [36.7%] or *Bombus* [33.3%]). *Tetralonia* was observed at all lowland sites. It was predominant on *Lespedeza cyrtobotrya* of NAM (36.7% of total observations), but not sighted in mountainous areas (NOG, PAN). This may be caused by preference of high temperature of *Tetralonia* because its distribution area are in subtropical to warm temperate regions in Asia. *Megachile* was constantly observed at all study sites and was the most frequent visitor for *Lespedeza formosa* subsp. *velutina* (53.1% of total observations at four sites). However, pollen load analyses revealed that two of five samples of *Megachile* had low proportions of Macrolespedeza pollen (29.0 and 32.3%: Table 3). This suggested that it sometimes showed lower constancy to Macrolespedeza than other apoid bees. *Bombus* was less frequently observed than *Apis, Tetralonia,* and *Megachile* but quite dominant at two sites, i.e., 45.5% of total visits to *Lespedeza melanantha* at PAN and 33.3% to *L. cyrtobotrya* at NAM. From these results, those four genera were supposed to be important flower visitors for pollination of Macrolespedeza at natural condition in Korea.

Among relatively minor flower visitors, *Nomia* (Halictidae) was slightly abundant at CHO (14.3% of all apoid records at the site). Pollen load analyses of *Nomia* on three samples were represented almost pure loads (94.9–100%) of Macrolespedeza pollen (Table 3), suggesting its constant visit to the Macrolespedeza flowers. Thus *Nomia* acts as one of effective pollinators at particular sites. The other apoid genera, such as *Coelioxys,* *Lasioglossum, Thyreus*, and *Xylocopa*, were neither frequently sighted nor locally abundant and considered to be less important for pollination of Macrolespedeza than previously mentioned genera. A visitor of Syrphidae (Diptera) was only observed on *Lespedeza melanantha* at PAN. Although its frequency was 18.2 % of total visits at the sites, it had a strong tendency to visit old flowers, which were already visited several times by other visitors, to feed remaining pollens from the anthers exposed from the keel-petals. Syrphid flies were, therefore, not effective pollinators even though they contacted to anthers or stigmas. Lepidopteran insects did not contact anthers or stigmas and were therefore solely "nectar thieves."

Discussion

Apoid bees as pollinators of Macrolespedeza species

From the present observation, we considered that apoid bees, especially *Tetralonia, Megachile*, and *Bombus*, were important flower visitors acting as pollinators of Macrolespedeza species in Korea. Frequent visitations of former two genera to Macrolespedeza species were also reported in previous studies in Japan. *Tetralonia mitsukurii* Cockerell, known as a highly oligolectic to Macrolespedeza species (Miyamoto 1961a); 90.6% of total visits to *Lespedeza cyrtobotrya* were made by the species in 6-year observations at Sasayama, Hyogo (Miyamoto 1962) and 93.0% of 2-year records at Itabashi, Tokyo (Tanaka 1980). It also showed high visitation frequency, i.e., 56.3% of total visits to two other Macrolespedeza species, *Lespedeza formosa* subsp. *velutina* (*L. bicolor* var *japonica*) at Sasayama, Hyogo (Miyamoto 1962) and 39.8% to *L. bicolor* at Setaura, Kumamoto (Iwata 1997). *Megachile* spp. are polylectic but predominantly visit to flowers of Leguminosae and Compositae (Miyamoto 1961b). *Megachile tsurugensis* Cockerell was second dominant visitor to *L. cyrtobotrya* and *L. formosa* subsp. *velutina* at Sasayama, Hyogo (Miyamoto 1962). *Megachile humilis* Smith and *M. nipponica* Cockerell also showed almost the same level of visitation frequencies as *M. tsurugensis* to *L. cyrtobotrya* (Miyamoto 1962). Therefore closer relationships between Macrolespedeza species and those two bee genera are similar between Korea and Japan and may be common in warm temperate parts within Macrolespedeza area.

In contrast to those two genera, predominant visitations of *Bombus* to Macrolespedeza species and supposed importance as a pollinator have not been mentioned so far. Relative frequencies of visitation by *Bombus* spp. were recorded in Japan to be 18.8% of total visits to *Lespedeza formosa* subsp. *velutina* at Sasayama, Hyogo (Miyamoto 1962) and 1.0% to *L. bicolor* at Setaura, Kumamoto (Iwata 1997), but no records on *L. cyrtobotrya* at Sasayama, Hyogo (Miyamoto 1962). These visitation frequencies were obviously lower than those of particular sites in Korea in this study. In the case of *Lespedeza melanantha* at PAN, the high frequency of *Bombus* visitation (45.5%) is related to its alpine habitat. *L. melanantha* is distributed in mountainous regions of more than 1100 m altitude in our observation. Proportion of *Bombus* in bee fauna has been known to be extremely high in alpine habitat, e.g., 76.9% of bee individuals were *Bombus* in Mt. Kushigata, Yamanashi, 35° 35'N, 1850–2050 m alt. (Kato et al. 1993) in comparison with 7.3% in Minami, Gifu, 35° 38'N, 400–500 m alt. (Yamauchi et al. 1976). In PAN, we observed that *Bombus* spp., mainly *B. ussurensis* Radoszkowski, *B. ignitus* Panfilov, and *B. modestus* Eversmann, were dominant flower visitors for various plants. Therefore, predominant visitation of *Bombus* to *L. melanantha* reflect faunal composition of its habitat.

In contrast to this case, 70.0% of *Bombus* to *L. cyrtobotrya* were recorded by *B. opulentus* Smith in lowland habitats sometimes close to the sea at NAM. The species was observed only at NAM in our expedition. According to Sakagami (1976), *B. opulentus* is one of the southern elements in the bumblebee fauna of Korea. Although *B. opulentus* is widespread and common in Korea (Sakagami 1976, Kim and Ito 1987), it is uncertain why the species was not observed at other lowland sites such as TAE. In KWA and CHO, however, decrease of floral diversity by human impacts and competition with naturalized European honeybees seem to be main causes of scarceness of bumblebees including *B. opulentus*. In contrast to solitary habits of *Tetralonia* and *Megachile, Bombus* is an eusocial bee and its colony is active from spring to autumn. Thus, it is necessary for establishment of bumblebee colonies that flowers are abundant and continuously bloom throughout the active season of bumblebees (Heinrich 1979). The decrease of floral diversity causes shortage of floral resources and disappearance of flowers during the active period of bumblebees. European honeybee is eusocial and collect resources from flowers much effectively than bumblebees. Competition with honeybees therefore strongly affect the survival of bumblebee colonies (Heinrich 1979, Roubik 1989).

Competitions among co-flowering plants including cultivated ones also may have significant effects on visitation frequencies of bumblebees. In TAE, for example, *Bombus ussurensis* and *B. ignitus* were abundant in our observation. However, they fully visited plants other than Macrolespedeza, for example, *B. ussurensis* visited *Impatiens textori* Miq. and *Melampyrum roseum* Maxim. and *B. ignitus* visited *Impatiens textori* Miq. (nectar robbing), *Serratula coronata* L. subsp. *insularis* (Iljin) Kitam., and cultivated *Tagetes patula* L. Both *Bombus* species were not observed on Macrolespedeza species at this site. Among the plants visited by *Bombus, Impatiens textori* and *Tagetes patula* were abundant and many *Bombus* workers visited intensively to flowers of them. Therefore, species composition and relative abundance of co-flowering plants may affect pollinator fauna of Macrolespedeza species, though confirmation should be needed by further observations and experimental manipulations.

Among bees less frequently observed than above genera, *Nomia* may have particular importance for Macrolespedeza pollination. In Japan, the most widespread species of *Nomia, N. punctata* Westood, is known as oligolectic to Leguminosae and frequently visit to *Lespedeza* species (Miyamoto 1960). *Nomia* may constitute one of major pollinators if the habitat are highly disturbed or isolated in small areas where eusocial bees difficult to colonize. In CHO where small hills surrounded by large urban area, eusocial bees other than European honeybee were not observed in spite of our extensive survey. At the site, *Nomia* may have considerable activity for Macrolespedeza pollination. The relative importance of *Nomia* to pollination for Macrolespedeza also needs further observations.

Relationship between flower visitor fauna and floral morphology of Macrolespedeza

In this study, we noted that relative visitation frequency of bees was different among Macrolespedeza species or in study sites. In our observation, however, differences of faunal composition surrounding Macrolespedeza species reflect a regional difference rather than a species-specific interaction between plants and insects. For example, *Lespedeza formosa* subsp. *velutina* was visited more frequently by *Megachile* (53.1% of total observations) at four sites than any other species observed, e.g., 36.4% of total observations on *L. melanantha*, 11.8% on *L. bicolor*, and 7.5% on *L.*

cyrtobotrya. In NOG and TAE, proportions of *Megachile* in total observations were also high on *L. bicolor* in NOG (100%) and on *L. cyrtobotrya* in TAE (50%). In NAM, both *L. cyrtobotrya* and *L. formosa* subsp. *velutina* showed higher proportions of *Tetralonia* than the averages, i.e., 36.7% (average: 18.2%) of total observations on *L. cyrtobotrya* and 80% (average: 21.9%) on *L. formosa* subsp. *velutina* were *Tetralonia*. We cannot find any significant differentiations of flower visitor fauna related to Macrolespedeza species except *L. melanantha*.

Lespedeza melanantha grows in the alpine regions in Korea. The predominance of *Bombus* is considered to be common for all populations, though we observed only a single site. According to Akiyama (1988), *L. melanantha* is close to *L. bicolor* and *L. cyrtobotrya*, but distinguished from the former by the wing longer than keel-petals and from the latter by the semicircular calyx-lobes. Our molecular phylogenetic analysis indicated the sister species relationship of *L. melanantha* and *L. bicolor* (Nemoto et al. in preparation). From a viewpoint of pollination biology, however, a reflected part of standard petal attract particular interests because it determine both a distance to nectary and a point of pollen deposition. When bees collect nectar and pollen from Macrolespedeza flowers, they first land on wings or keel-petals and put their heads at the reflected part of standard petals. Then they elongate glossa to the basal portion of androecia for collecting the nectar and push down the keel petals to collect the pollen. Therefore, length from the base to the reflected part of standard (LBR hereafter) is related to length of the glossa of flower visitors. On the one hand, length from the reflected part to the top of keel-petals (LRK in following parts) is that of "landing platforms" for flower visitors and length from the reflected part to anthers (almost the same as LRK) is regarded as to determine the point of pollen deposition to visitor bodies. According to Akiyama (1988), standard of *L. melanantha* are narrower than *L. bicolor* and reflected at the part about 1/2 the way from the base, whereas those of *L. bicolor* are wider and reflect at the part about 1/3 from the base. Therefore the LBR of *L. melanantha* is longer than that of *L. bicolor*, i.e., ca. 5 mm in *L. melanantha* and 3–4 mm in *L. bicolor* calculated from the data of Akiyama (1988). Also in our observation, the basal half of standard of *L. melanantha* surrounded wings and keel-petals and showed a tubular form. On the other hand, *L. melanantha* is shorter in the LRK than *L. bicolor*, i.e., ca. 4 mm in *L. melanantha* and 5–6 mm in *L. bicolor* calculated from the data of Akiyama (1988). Therefore *L. melanantha* have more tubulous flowers with smaller "platform" than *L. bicolor*.

In this study, we observed that the most frequent flower visitor of *L. melanantha* was *Bombus* and its visitation frequency was higher than any other bees. Although there is extreme inter- or intraspecific variation in glossa length (e.g., Plowright and Caverty 1984, Inoue and Kato 1992), *Bombus* have the longest glossa among major flower visitors of Macrolespedeza (5.8–14.5 mm in workers compare with *Megachile* [5.1–7.4 mm in females and 3.3–4.5 mm in males], *Apis* [3.5–5.3 mm in workers] and *Tetralonia* [2.4–3.8 mm]: based on Taniguchi [1954] at Sasayama, Hyogo, Japan). In contrast to other bees, *Bombus*, for its large body size, hook its legs and hang itself to keel-petals instead of landing on them when it collect nectar and pollens from small legume flowers. *Bombus* individuals often collect nectar not from the front of flowers but form side of them using their long glossa. When they access to nectar from the side of flowers, they do not have enough contact to anthers or stigmas. Thus papilionaceous flowers with short LBR tend to be robbed their nectar by *Bombus*. The tubuliform flower of *L.*

melanantha may evolved suffering the selective pressure of nectar robbing. Tubulous flowers of legume have a function to restrict pollinator visitations only form the front of them and such direction of flower evolution also known in bumblebee-pollinated *Trifolium* (Arroyo 1981). More comprehensive studies including pollination efficiency of each flower visitor should be necessary to confirm the direction of the evolution.

There are also considerable differences in flower form among Macrolespedeza species observed in this study. According to Akiyama (1988), *L. cyrtobotrya* also have longer LBR than *L. bicolor* and flowers of *L. formosa* subsp. *velutina* are the longest LRK among the four examined in the present study. In this study, however, we cannot find any particular relationships between flower forms and flower visitor fauna among three species, *L. cyrtobotrya, L. bicolor*, and *L. formosa* subsp. *velutina*. More observations and experiments should be needed to clarify whether these differences in flower form are results of selection by flower visitors.

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韓国産マメ科ハギ属ヤマハギ亜属の訪花昆虫に関する予察的観察

マメ科ハギ属ヤマハギ亜属はメドハギ亜属に比べて大きな花をつけ閉鎖花をもたないことから，昆虫媒であると考えられている．しかし，これまでの訪花昆虫に関する報告は散発的で，送粉に有効な訪花昆虫の確認，ヤマハギ亜属種間における訪花昆虫相の比較などについて基本的な調査を行うべき点が多い．本報では，1997年に行った韓国での野外調査の際に，4種のヤマハギ亜属植物について訪花昆虫の観察と訪花昆虫相の比較を行った結果を報告する．韓国産ヤマハギ亜属に関する訪花昆虫の観察は，本研究がはじめてである．主要な訪花昆虫は4属ともハナバチ類で，特にミツバチ属（*Apis*），ヒゲナガハナバチ属（*Tetralonia*），ハキリバチ属（*Megachile*），マルハナバチ属（*Bombus*）の頻度が高かった．訪花昆虫のもつ花粉の分析から，ハナバチ類はヤマハギ亜属に対する高い定花性を示すことがわかり，重要な送粉昆虫となっていると推測した．観察した4種のうち3種の間では訪花昆虫相はほぼ類似していたが，クロバナキハギ（*Lespedeza melanantha*）はマルハナバチ属が優占する点で他の3種と著しく異なっていた．ヤマハギ亜属でマルハナバチ属が優占して訪花するとの報告はこれまでなかったが，クロバナキハギでは最も重要な送粉昆虫となっていた．マルハナバチ属はヤマハギ亜属に訪花が観察されたハナバチ類の中では最も中舌長が長く，クロバナキハギが他の種に比べて旗弁の舷部が短く筒状の花を持つことは，このことと関連があると考えられる．

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